

## AMENDMENTS TO THE CLAIMS

*A listing of the claims presented in this patent application appears below. This listing replaces all prior versions and listing of claims in this patent application.*

### **Claims 1-12 (canceled).**

**Claim 13 (previously presented):** A biosensor for the detection and/or the determination of biogenic amines as freshness markers, comprising:

an electrode and

- (i) a mono-enzyme system of an amine oxidase or
- (ii) a bi-enzyme system of an amine oxidase and a peroxidase,

wherein said amine oxidase is a copper-containing grass pea oxidase (E.C. 1.4.3.6) and said electrode is a carbon/graphite based electrode, and whereby said amine oxidase is cross-linked to the electrode into an osmium based redox polymer.

**Claim 14 (previously presented):** The biosensor according to claim 13, characterised in that the bi-enzyme system contains said copper-containing amine oxidase derived from grass pea coupled with horseradish, soybean, tobacco, sweet potato or palmtree peroxidase.

**Claim 15 (previously presented):** The biosensor according to claim 14, characterised in that the peroxidase is horseradish peroxidase.

**Claim 16 (previously presented):** The biosensor according to claim 13, characterised in that the osmium based redox polymer includes poly(1-vinylimidazole) complexed with  $[\text{Os}(\text{4,4'-dimethyl-bi-pyridin})_2 \text{Cl}]^{+/2+}$  and poly(ethyleneglycol)diglycidyl ether, as the crosslinking agent.

**Claim 17 (previously presented):** The biosensor according to claim 13, characterised in that the biosensor is of Type I, Type II or Type III type of biosensor, wherein:

Type I: the mono-enzyme or the bi-enzyme system is added direct onto the electrode surface; or

Type II: the mono-enzyme or the bi-enzyme system is entrapped in the osmium based redox polymer added on the surface of the electrode; or

Type III: the mono-enzyme or the bi-enzyme system and the osmium based redox polymer forms sequential coatings added on the surface of the electrode.

**Claim 18 (previously presented):** The biosensor according to claim 17, characterised in that the biosensor of Type III is one of Type III a, Type III b, Type III c or Type III d, wherein:

Type III a: a second coating of the mono-enzyme is coating a dried layer of peroxidase and redox hydrogel; or

Type III b: a second coating of peroxidase and redox hydrogel is coating a dried layer of the mono-enzyme; or

Type III c: a second coating of the mono-enzyme entrapped in redox hydrogel is coating a dried layer of peroxidase; or

Type III d: a second coating of peroxidase is coating a dried layer of mono-enzyme entrapped in redox hydrogel.

**Claim 19 (previously presented):** A biosensor according to claim 15, wherein the weight ratio of amine oxidase to horseradish peroxidase is 80:20.

**Claim 20 (currently amended):** A method for the detection or determination of freshness biomarkers or of the content of freshness biomarkers in a food sample, comprising the steps of establishing a standard curve between the electrical output of the biosensor of claim 13 and freshness, wherein said freshness biomarkers are biogenic amines, applying said sample to the biosensor of claim 13, detecting an electrical output from said biosensor, and comparing the

electrical output of the biosensor when applied to the sample with said standard curve for freshness biomakers to detect and determine the freshness of the food sample ~~to some standard curve for amines or for histamine; establishing a relationship between the electrical output and freshness or for histamine; and applying said sample to the biosensor of claim 13 and detecting an electrical output from said biosensor.~~

**Claim 21 (currently amended):** A method for the detection or determination of histamine in a body fluid sample, in medical diagnoses or in the treatment of a disease ~~from a sample~~, comprising the steps of establishing a standard curve between the electrical output of the biosensor of claim 13 and histamine, applying said sample to the biosensor of claim 13, detecting an electrical output from said biosensor, and comparing the electrical output of the biosensor when applied to the sample with said standard curve for histamines to detect and determine the histamines of the food sample ~~to some standard curve for amines or for histamine; establishing a relationship between the electrical output and freshness or for histamine; and applying said sample to the biosensor of claim 13 and detecting an electrical output from said biosensor.~~

**Claim 22 (previously presented):** A method for the detection or determination of histamine in a sample of microdialysates or dialysates, comprising the steps of applying said sample to the biosensor of claim 13 and detecting an electrical output from said biosensor.

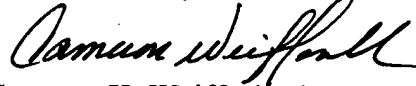
Application No. 10/019,651

This Second Supplemental Amendment Under 37 CFR § 1.116 is being filed to reduce the issues on appeal. The Amendment amends claims 20 and 21 as suggested by the Examiner in the Advisory Action dated January 5, 2004. If the amendment is entered, the rejection of claims 20 and 21 under 35 U.S.C. § 112, second paragraph, as set forth in the Advisory Action should be overcome. Accordingly, it is respectfully requested that Amendment be entered.

To the extent necessary, a petition for an extension of time under 37 C.F.R. 1.136 is hereby made. Please charge any shortage in fees due in connection with the filing of this paper, including extension of time fees, to Deposit Account 500417 and please credit any excess fees to such deposit account.

Respectfully submitted,

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